RELATIVE BIOAVAILABILITY OF OMEPRAZOLE CAPSULES AFTER ORAL DOSING

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ABSTRACT

Omeprazole, a proton pump inhibitor, effectively suppresses the gastric acid secretion in the parietal cells of stomach. Pharmacokinetics and relative bioavailability of generic products of omeprazole were compared with innovator product, Losec. Twelve healthy adult volunteers participated in the study which was conducted according to a randomized, open-label single dose Latin square cross over design. The preparations were compared using area under the plasma concentration – time curve (AUC), peak plasma concentration (C_{max}), and time to reach peak plasma concentration (t_{max}). The two generic capsules proved to be bioequivalent with brand-name omeprazole with regard to the pharmacokinetic parameters C_{max}, AUC_{0-t}, AUC_{0-inf} and t_{max}. Moreover the parametric confidence intervals (90%) for the ratio of the C_{max}, AUC_{0-8} and $AUC_{0-\infty}$ values lie between 0.8-1.2. The test formulations were found bioequivalent to the reference formulation by the one-way ANOVA test procedure. On the basis of these results, the 3 formulations were considered to be bioequivalent. Two subjects demonstrated increase in AUCs and high C_{max} after administration of either product which may attribute to the ethnic disposition of omeprazole in these subjects.

Keywords: Omeprazole, Bioequivalence, Pharmacokinetics, Ethnic Disposition

INTRODUCTION

Omeprazole, a gastric acid pump inhibitor which has greater anti-secretary activity than histamine H_2 receptor antagonists has been widely used in the treatment of reflux oesophagitis, Zollinger–Ellison syndrome and peptic ulcer disease (1,2). In order to prevent degradation of drug in acid media, the drug is formulated as enteric-coated granules in capsule forms. Differences in the quality of the granules coating are a potential limiting factor for in vivo performance of the product and various product may cause different bioavailability parameters. Furthermore, the mean time to attain maximum plasma concentrations (t_{max}) of omeprazole is highly formulation dependent (3). It is a very well tolerated drug and its doses are 20 mg up to 80 mg (4). Omeprazole terminal half-life is between 0.5 and 2 hours (5-8). Although omeprazole is well absorbed from the gastrointestinal tract, its oral bioavailability in humans is about 40 to 50% suggesting pronounced first pass metabolism for this drug (4).

Omeprazole is eliminated rapidly and almost completely by liver metabolism. After absorption, it is metabolized and 3 main metabolites; omeprazole sulphone, omeprazole sulphide and hydroxy omeprazole have been identified in human plasma (8-10). Hydroxylation of omeprazole at the 5-position is subject to genetic polymorphism and the sulphone in plasma is cumulated in poor metabolizers of S-mephenytion 4' hydroxylation (11). Therefore, the majority of individuals metabolize the drug normally, and only a small number might be expected to be poor metabolizer (11). Clinical experiences with omeprazole has been gained for more than 20 years of its clinical use (1,2,4, 5,8,12,13). Various studies have investigated the pharmacokinetic properties of omeprazole (1-5,11-13), however increasing requirements for proof of pharmacokinetic data make new studies mandatory to confirm earlier findings according to today's standards. Thus, the aim of this study was to determine pharmacokinetics and relative bioavailability of omeprazole in man following oral administration of omeprazole enteric-coated granules in capsules.

MATERIALS AND METHODS

Commercial oral dosage forms of omeprazole 20mg enteric coated granules in capsule were provided by two Iranian pharmaceutical manufacturing companies, Abidi (omeprazole) and lorestan (lorsec). Losec®, a reference product, was bought from astra Sweden. Omeprazole powder was provided by the Abidi Pharmaceutical Co. Flunitrazepam was a gift from the pharmacology laboratory of our faculty. All other chemicals and reagents were HPLC or analytical grade.

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Table 1. Pharmacokinetic parameters of omeprazole in ten normal metabolizer of omeprazole (mean± S.D.)

Treatment	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-8} (ng.h/ml)	AUC_{0-inf} (ng.h/ml)	$T_{1/2}$ (h)
Omeprazole	283 ± 113	1.75 ± 0.63	455 ± 155	481 ±175	2.04 ± 0.82
Lorsec®	276 ± 94	2.40 ± 0.88	489 ± 180	503 ± 175	1.82 ± 0.68
Losec®	284 ± 105	1.60 ± 0.57	461 ± 171	487 ± 161	1.96 ± 0.71
CI for Omeprazole	0.86-1.17	N.R	0.88-1.11	0.87-1.13	N.R
CI for Lorsec®	0.86-1.14	N.R	0.99-1.15	0.95-1.13	N.R

CI = 90% Confidence Interval, NR = Not Required, C_{max} = Maximum plasma concentrations, T_{max} = Time required to reach the maximal concentrations, AUC_{0-8} = AUC until last quantified sample using the trapezoidal rule, AUC_{0-inf} = The total AUC until infinity, $T_{1/2}$ = Terminal half life

Table 2. Pharmacokinetic parameters of omeprazole in subjects 7 and 9.

Treatment	C_{max} (ng/ml)		T_{max} (h)		AUC_{0-8} (ng.h/ml)		AUC_{0-inf} (ng.h/ml)		$T_{1/2}$ (h)	
	7	9	7	9	7	9	7	9	7	9
Omeprazole	676	923	4	3	2597	1720	4853	1930	4	1.5
Lorsec®	628	457	3	4	2793	1032	4848	1050	5	1.2
Losec®	991	899	1	1.5	3876	2075	4685	2125	3	1.4

C_{max} = Maximum plasma concentrations, T_{max} = Time required to reach the maximal concentrations, AUC_{0-8} = AUC until last quantified sample using the trapezoidal rule, AUC_{0-inf} = The total AUC until infinity, $T_{1/2}$ = Terminal half life

Study design
The study was based on a single-dose, Latin square cross over design under fasting condition. After an overnight fasting (for 10 hours) subjects were given one capsule of either product followed by 250 ml of water. They were fasted over 3 hours post-doses and then they received the same breakfast and lunch according to the time scheduled. Therefore, all subjects received equivalent of 20 mg omeprazole on three occasions separated by a 7 days wash out period.

Volunteers
Twelve healthy Iranian male subjects participated in the study. The ages of subjects were between 22 and 24 years (mean age ± SD, 23.6 ± 0.7 years). The average body weight was 76.25 ± 8.4 kg (range 62.0 – 87.0kg) and the average height was 178.5 ± 3.68 cm (range 172-186 cm). Prior to inclusion into the study, written informed consent of each subject was obtained. The purpose, the nature of the study and any possible risks were explained and it was made clear, that any subject may withdraw voluntarily from the study at any time without prejudice. Before the beginning of the trial a detailed medical and clinical-chemical examination of all volunteers was carried out, which revealed normal finding in all examination. Twelve hours before medication and during the study, all subjects abstained from caffeine containing foods and drinks, and nicotine. No medication was allowed one week before and during the study.

Blood sampling
10 ml blood samples were taken from a cubical vein into heparinized tubes at the following time points: 0 h (prior to administration), and at 0.5, 0.75, 1, 1.5,2, 2.5, 3, 4, 5, 6, 8 hour following dosing. Blood samples were centrifuged within 15 min and the plasma stored at - 20ºC until analyzed.

Omeprazole analysis
Analysis of omeprazole in plasma was performed using a validated high performance liquid chromatographic assay (6) with some modifications. To 1ml of the plasma sample was added, 100 μl of methanol: acetate buffer (pH=9.6) (1:4v/v) and after mixing with 5ml of dichloromethane: acetonitrile (4:1v/v) it was vortexed for 30 seconds. Following centrifugation at 2000g for 10 min, 4 ml of the organic phase was separated and evaporated under a nitrogen stream. The residue was dissolved in 200μl of mobile phase, and 100 μl was injected into the HPLC system consisting of a reversed-phase. Nova-pack C_8 (15cm x 3.0mm, 4μm, waters), column which was maintained at room temperature. The UV detector was set at 302 nm. The mobile phase was a mixture of methanol: acetonitrile: phosphate

buffer (pH 7.2) (40:8:52,v/v) and was pumped at a flow rate of 1ml/min. Quantitation was obtained by calculation of the peak area ratio of omeprazole to the internal standard. The values of coefficient variation were 3.15% at 100 ng/ml and 3.99% at 10 ng/ml (n=9). The lower limit of quantitation was 5 ng/ml.

Pharmacokinetic data analysis
The AUC was calculated by the linear trapezoidal rule. The area from the last concentration point (C_{last}) to infinity was calculated as C_{last}/β, where β was the terminal elimination rate constant calculated by regression through at least three data points in the terminal elimination phase. The terminal elimination half-life ($t_{1/2}$) was calculated by $0.693/\beta$. Maximum plasma concentrations (C_{max}) and the time required to reach the maximal concentrations (t_{max}) were obtained directly from plasma concentrations versus time curve of each individual volunteers.

Statistical analysis
Pharmacokinetic variables and bioequivalence metrics from each study were compared using analysis of variance (ANOVA). The ANOVA model included sequence, subject nested within sequence, phase and treatment (omeprazole, lorsec® and losec®) as factor. After logarithmic transformation C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were analyzed according to the current FDA guidelines (14). The 90% confidence interval of the ratio of the test / reference (T/R) was calculated according to the reported methods (15,16). In all tests, a probability level of significance preset at $\alpha = 0.05$. All statistical analysis was performed using SPSS 10.

RESULTS
Inspection of the omeprazole pharmacokinetic data revealed that subjects 7 and 9 eliminated omeprazole slowly. Therefore, the data of these subjects were excluded from the statistics and are presented separately. The pharmacokinetic results of three different oral formulations of omeprazole are summarized in table 1.
Figure 1 depicts the mean plasma concentrations of the group of 10 subjects with normal metabolic status. Fig 2 and 3 show the plasma concentrations of subjects 7 and 9. The 90% confidence intervals of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ are summarized in table 1 as well. After administration of the test products, peak plasma concentrations of 283 ± 113 and 276 ± 94 ng/ml were obtained for omeprazole and lorsec formulations respectively. The corresponding value after administration of the reference capsule (Losec®) was 284 ± 105. The statistical analysis did not show any significant differences for C_{max} in

three formulations. The 90% confidence intervals of this value were in the ranges 0.86-1.17% for omeprazole and 0.86-1.14 for lorsec respectively.
The $AUC_{0-\infty}$ was calculated to be 481 ± 175 ng.h/ml for omeprazole, 503 ± 175 ng.h/ml for lorsec and 487 ± 161 ng.h/ml for losec. The estimated relative bioavailability amounted to 1 ± 0.2 % and 1.04 ± 0.25% for omeprazole and Lorsec® respectively. Statistical analysis showed equivalency of both dosage forms with the 90% confidence interval of 0.87-1.13 for omeprazole and 0.95-1.13 for Lorsec. Similar finding were also observed for AUC_{0-t} (omeprazole 455 ± 155 ng.h/ml, Lorsec 489 ± 180 ng.h/ml, and losec 461 ± 171), relative bioavailability and 90% confidence intervals for omeprazole and Lorsec® respectively. The $AUC_{0-\infty}$ and AUC_{0-t} for the three products were not statistically different ($p > 0.05$).
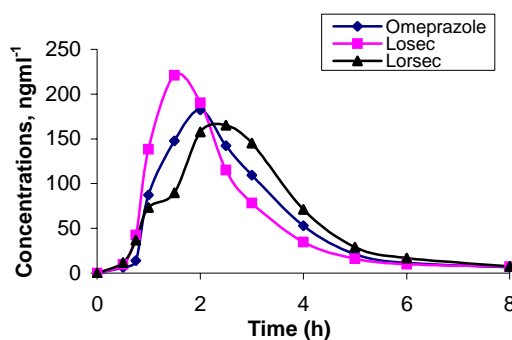

Fig. 1. Omeprazole plasma concentrations in 10 normal metabolizers following single oral administration of 20 mg omeprazole in enteric coat granulated capsules.

After log transformation of AUC and C_{max} no statistical significant were found. A statistically significant difference was observed between the T_{max} values ($p < 0.044$) of the two products. The terminal half-life was not different in these products significantly.

DISCUSSION
The aim of the present study was to assess the relative bioavailability of two enteric-coated granulated omeprazole capsules in comparison to a reference product, Losec®.
The plasma levels and pharmacokinetic data revealed that two subjects (7 and 9) may be poor metabolizers of omeprazole as the AUC were approximately 2-6 times greater in these subjects. The pharmacokinetic data of these subjects were therefore excluded from the biometrical analysis and are discussed separately (Table 2).
Omeprazole was safe and well tolerated by all subjects. None of the subjects reported any adverse events that could be related to the medication. It should be emphasized that subjects 7 and 9 did not
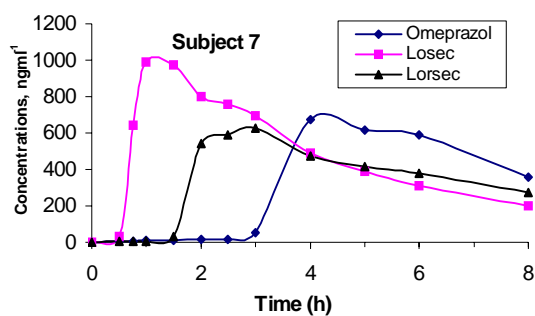
Fig. 2. Omeprazole plasma concentrations in subject 7 following single oral administration of 20 mg omeprazole in enteric coat granulated capsules.
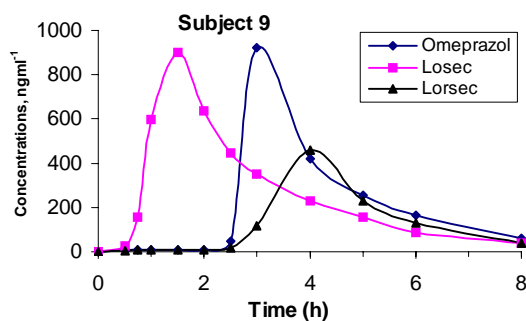

Fig. 3. Omeprazole plasma concentrations in subject 9 following single oral administration of 20 mg omeprazole in enteric coat granulated capsules.

experience any adverse drug reactions during the study.

The AUC_{0-t} and $AUC_{0-\infty}$ for the three products were not statistically different ($p>0.05$) suggesting comparable plasma profiles for these products.

After log transformation, ANOVA showed no statistical differences between three formulations as well. The statistical analysis did not show any considerable differences in periods, formulations or sequences ($p>0.05$). On the basis of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$, the capsules fulfilled the formal criteria for bioequivalency to the reference product. For $AUC_{0-\infty}$, the treatment ratio were estimated to be 1 ± 0.2 % and 1.04 ± 0.25% for omeprazole and lorsec respectively, indicating complete bioavailability of omeprazole from the test products in comparison to the registered product losec$^{®}$. Similar results were obtained for AUC_{0-t} of the treatment ratio. T_{max} demonstrated the expected delay of the absorption from the enteric-coated granulated capsules. A statistically significant difference were observed between the T_{max} values ($p <0.044$), although from the therapeutic point of view the slight differences may not be significant or important. The pharmacokinetic findings in this study are well in agreement with published data for earlier trials (2,17). Although in other investigations (17) the confidence interval of C_{max} for their products fell outside the FDA accepted range (0.8-1.25%). These values in our study were between the accepted ranges. The differences that they have found in C_{max} may be the results of having some subjects who are poor metabolizes since these authors did not exclude them from their data. The disposition kinetic of omeprazole has been studied specifically in extensive and poor metabolizers of S-mephenytion and pronounced inter-phenotypic differences ($P<0.001$) between the two groups with

regard to the mean kinetic parameters of omeprazole including T_{max} has been described

Furthermore, it is reported (11) that the $t_{1/2}$ and mean AUC value were approximately 3 times longer and 10 times greater in poor metabolizers than in extensive metabolizers. The deficient metabolizers are known to build up high plasma concentrations over longer periods of time, and have increased elimination half-lives and T_{max}. Our results showed that the mean AUC values were approximately 2-6 times greater in two subjects. The half-life of omeprazole however was not different in these subjects which might be due to the time of sample collection since samples were taken only for 8 hours.

The findings that two subjects out of twelve Iranian volunteers might be poor metabolizers of omeprazole is somewhat surprising, since it is well known that the frequency of occurrence of the poor metabolizer phenotype of S-mephenytoin is much greater (17-23%) in oriental (18-20) than that of Caucasian (3-6%) populations (21-23). This might be due to the number of subjects that participated in this study.

In conclusion, the pharmacokinetic results of this study confirm earlier findings and demonstrate complete bioavailability of the marketed capsules compared to the reference product. The results of this study also emphasize that it is advisable to assess the metabolic status by phenotyping subjects with an adequate test prior to conducting pharmacokinetic studies.

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